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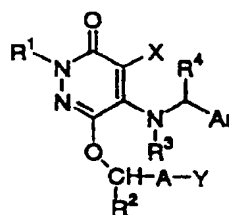
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权利要求书 6 页 说明书 24 页 附图页数 2 页

[54] 发明名称 勃起功能障碍治疗剂

[57] 摘要

本发明提供一种勃起功能障碍的治疗剂, 该治疗剂包括以式 (I) 表示的 3(2H) - 哒嗪酮衍生物或其药用盐作为活性成分, 其中的各个符号与说明书中的定义相同。



(I)

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C₁₋₄ 烷基或卤原子取代的苯基， 或表示 $S(O)_m-R^{12}$ ， 其中 m 是 0-2 的整数和 R¹² 表示 C₁₋₄ 烷基。

5. 权利要求1-3任意一项所述的勃起功能障碍治疗剂， 其中Ar是吡啶基、N-氧化吡啶基或被OR²² 取代的苯基， 其中R²² 表示C₁₋₄ 烷基。

5 6. 权利要求1-5任意一项所述的勃起功能障碍治疗剂， 其中A是直链或支链的C₁₋₈亚烷基， 其中直链上的碳原子可被一个羟基（-OH）取代。

7. 权利要求4-6任意一项所述的勃起功能障碍治疗剂， 其中Y是下式表示的苯基：



其中 R¹⁰ 和 R¹¹ 各自独立地表示氢原子， 卤原子， C₁₋₄ 烷基， C₁₋₄ 酰氨基， OR⁵， 其中 R⁵表示氢原子或 C₁₋₄ 烷基， NHSO₂R⁹， 其中 R⁹表示被氢原子、C₁₋₄ 烷基或卤原子取代的苯基， 或表示 $S(O)_m-R^{12}$ ， 其中 m 是 0-2 的整数和 R¹² 表示 C₁₋₄ 烷基。

15

8. 权利要求7所述的勃起功能障碍治疗剂， 其中Y是被卤原子取代的苯基。

9. 权利要求5-8任意一项所述的勃起功能障碍治疗剂， 其中Ar是3-吡啶基基团。

20 10. 权利要求1所述的勃起功能障碍治疗剂， 其中式（I）表示的化合物是4-溴-6-[3-(4-氯苯基)丙氧基]-5-(3-吡啶基甲基氨基)-3-(2H)-哒嗪酮或4-溴-6-[3-(4-氯苯基)-3-羟基-丙氧基]-5-(3-吡啶基甲基氨基)-3-(2H)-哒嗪酮， 或其药用盐。

温和的。

本发明的哒嗪酮化合物(I)具有 PDE III 和 PDE V 两种抑制活性，可用作勃起功能障碍的治疗剂，与本领域的治疗方法相比其应用得到改进。

Inhibition of allergen-induced lung eosinophilia by type-III and combined type III- and IV-selective phosphodiesterase inhibitors in Brown-Norway rats

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Abstract. We examined the effect of a type IV (rolipram) and a combined type III and IV (Org 20421) isoenzyme-selective phosphodiesterase inhibitor upon allergen-induced pulmonary eosinophil recruitment in sensitised Brown Norway rats. Rats were sensitised with ovalbumin intraperitoneally and later challenged with ovalbumin aerosol which induced a significant increase in the total eosinophil and neutrophil count in bronchoalveolar lavage fluid at 24 hours (from 0.38 ± 0.12 to $1.36 \pm 0.18 \times 10^6$, $p < 0.01$ and from 0.06 ± 0.01 to $0.33 \pm 0.07 \times 10^6$, $p < 0.01$) respectively. Pretreatment with rolipram ($30 \mu\text{mol/kg}$) and Org 20421 ($30 \mu\text{mol/kg}$) abolished the eosinophilia and neutrophilia evoked by ovalbumin. We conclude that type IV and possibly type III isozyme phosphodiesterase inhibitors may regulate, directly or indirectly, eosinophil and neutrophil activity and/or those cells responsible for attracting them into the lung.

Key words: Org 20421 – Rolipram – Eosinophilia – Asthma – Brown-Norway rat

Introduction

Pulmonary eosinophilia is a feature of asthma, and eosinophils have been implicated in the pathogenesis of epithelial cell damage and bronchial hyperresponsiveness observed in asthma [1]. The mechanisms underlying the recruitment of eosinophils to the lungs are unclear but recent evidence suggests that this may be partly dependent on activated CD4⁺ T-lymphocytes of the Th-2 subset and the subsequent release of cytokines including IL-3, IL-5 and GM-CSF [2].

Cyclic nucleotides regulate the activity of many cells in the airways, including proinflammatory immunocompetent cells such as macrophages, eosinophils, mast cells and lymphocytes [3]. Cyclic purine phosphodiesterases

(PDE) are responsible for the inactivation of cyclic 3',5'-adenosine monophosphate (cAMP) and cyclic 3',5'-guanosine monophosphate (cGMP). At least seven PDE isoenzyme families have now been characterised [4, 5] which hydrolyse cAMP and/or cGMP, representatives of which are differentially expressed between cells and tissues. In inflammatory cells, the predominant PDE's expressed are the types III and IV isoenzymes [6]. *In-vitro* studies have established that inhibition of these isoenzymes by selective PDE inhibitors suppresses various aspects of cell activation [3, 7–9].

To determine whether selective PDE inhibitors possess inhibitory activity *in vivo*, we examined the ability of a type IV PDE isoenzyme inhibitor, rolipram [10] and of a combined Type III and IV isoenzyme inhibitor Org 20421 [11] to inhibit allergen-induced eosinophil accumulation into the lungs of ovalbumin-sensitised Brown Norway rats. This species was chosen because it mimics several of the features characteristic of asthma, including the recruitment of eosinophils to the lungs following allergen challenge [12].

Materials and methods

Sensitisation procedure and allergen exposure

Inbred female Brown Norway rats (weight: 180–200 grams) were sensitised by intraperitoneal (ip) injection of 1 ml of a suspension of 1 mg ovalbumin (OA) and 100 mg of aluminium hydroxide ($\text{Al}(\text{OH})_3$) in 0.9% (wt/vol) saline given on three successive days. Seventeen to twenty-one days after the initial intraperitoneal injection, rats were exposed to an aerosol of either 1% OA or saline and 18–24 hours later, they were anaesthetised and bronchoalveolar lavage fluid collected.

Protocol

Four groups of sensitised rats were used:

Group A (control – saline aerosol $n = 7$). 17–21 days after sensitisation, rats were injected i.p. with the first of three placebo

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(DMSO) injections given at 24 h, 6 h and 0.5 h prior to a single saline aerosol exposure for 15 min. Rats were studied 18–24 hours later.

Group B (placebo – allergen aerosol, $n = 8$). Same as Group B above, except that the rats were exposed to a 1% ovalbumin aerosol for 15 min.

Group C (rolipram – allergen aerosol, $n = 8$). 17–21 days after sensitisation, rats were injected i.p. with rolipram (30 $\mu\text{mol/kg}$) administered at 24 h, 6 h and 0.5 h prior to single allergen aerosol exposure for 15 min. Rats were studied 18–24 h later. This dose of rolipram has previously been shown to prevent antigen- and mediator-induced bronchoconstriction [13, 14].

Group D (Org 20241 – allergen aerosol, $n = 8$). Same as Group C, except that Org 20241 (30 $\mu\text{mol/kg}$) was administered instead.

Aerosol exposure

Aerosol exposure was accomplished by placing the rats in a 6.5 litre plexiglass chamber. This was connected to a Devilbiss Pulmosonic nebuliser (Model No 2512, Feltham, Middlesex, UK) which generated an aerosol mist which was pumped into the exposure chamber by the airflow supplied from a small animal ventilator set at 10 strokes min^{-1} with a pumping volume of 10 ml.

Bronchoalveolar lavage

Rats were given an overdose of sodium pentobarbitone (100 mg/kg iv) and the lungs were lavaged with $10 \times 2 \text{ ml}$ aliquots of 0.9% w/v sterile saline via a polyethylene tube through an upper tracheostomy site. Lavage fluid was centrifuged ($\times 500 \text{ g}$ for 10 min at 4°C) and the cell pellet was resuspended in 0.5 ml of Hank's balanced salt solution. Total cell counts were made by adding 10 μl of the cell suspension to 90 μl of Kimura stain and counted in a Neubauer chamber under a light microscope. Differential cell counts were made from cytopsin preparations stained with May-Grunwald stain. Cells were identified as eosinophils, lymphocytes, neutrophils and macrophages by standard morphology and 500 cells were counted under $\times 400$ magnification. The percentage and absolute number of each cell type were calculated.

Data analysis

Data are reported as mean \pm SEM. Non-parametric analysis of variance (Kruskal-Wallis method) was used to determine significant variance among the groups. If a significant variance was found, Mann-Whitney U-test was used to analyse for significant differences between individual groups. $p < 0.05$ was considered to be significant.

Results

Aerosol exposure

Rats sensitised by i.p. injection of OA responded when exposed to 1% OA aerosol solution by showing obvious respiratory distress characterised by the development of a defensive posture and exaggerated laboured breathing movements. This immediate response started within 2–4 mins and all animals recovered spontaneously. This response was not inhibited by pretreatment of the rats with either rolipram or Org 20241.

Cellular content of BAL fluid

There was a significant increase in total numbers of

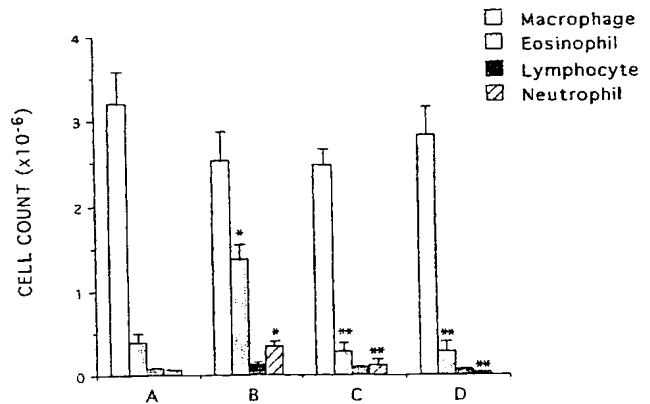


Fig. 1. Total macrophage, eosinophil, lymphocyte and neutrophil counts (from left to right) in sensitised Brown-Norway rats exposed to saline (group A), exposed to ovalbumin (OA) (group B) and exposed to OA but pretreated with rolipram (group D) or Org 20241 (group C). Data presented as mean \pm SEM. * $p < 0.01$ compared to group A and ** $p < 0.001$ when compared to group B.

eosinophils and neutrophils in BAL fluid after placebo pretreatment (Group 2) when compared to control rats (Group 1), from 0.38 ± 0.12 and 0.06 ± 0.01 to 1.36 ± 0.18 and $0.33 \pm 0.07 \times 10^6$, respectively. There was no significant change in macrophage or lymphocyte count. The increase in the number of neutrophils and eosinophils was significantly inhibited by pretreatment of rats with both rolipram and Org 20241 to levels similar to those observed in control animals (Group A) (Fig. 1). The eosinophil count after Org 20241 and rolipram was 0.28 ± 0.11 and $0.27 \pm 0.13 \times 10^6$ respectively ($P < 0.001$ compared to placebo treatment). Org 20241 and rolipram reduced neutrophil numbers to 0.12 ± 0.03 and $0.03 \pm 0.01 \times 10^6$ cells respectively ($P < 0.001$) (Fig. 1).

Discussion

We have shown that rolipram, a type IV PDE inhibitor, and Org 20241, a mixed inhibitor of the PDE III and PDE IV isozyme families, suppressed the infiltration of eosinophils into bronchoalveolar lavage fluid of sensitised Brown-Norway rats in response to ovalbumin provocation. In addition, the influx of neutrophils was also inhibited. Our data thus support a potential anti-inflammatory role for PDE IV inhibitors in this species; a role for PDE III isozyme cannot be excluded. Similar data have been reported for rolipram [14], zardaverine [15] and benzafertrine [16, 17] in the guinea-pig. The importance of our findings is that inhibition of eosinophilia and neutrophilia is demonstrated in an animal model of asthma that mimics closely in many respects asthma in man. Unlike the guinea-pig, Brown-Norway rats are IgE-producing animals and corticosteroid-sensitive [18, 19].

The mechanisms underlying the accumulation of eosinophils in the airways following allergen challenge are unclear. This accumulation may depend upon activation of T-lymphocytes to release factors that may

induce the recruitment and prolonged survival of eosinophils, such as the cytokines interleukin (IL)-3, IL-5 and GM-CSF [2]. We have previously shown that there is an increase in CD25⁺ lymphocytes in bronchoalveolar lavage fluid in sensitised Brown-Norway rats challenged with ovalbumin [20]. In previous studies, glucocorticosteroids have also been shown to inhibit eosinophil accumulation in this model [18]; in addition, a more specific inhibitor of activated T-lymphocytes, cyclosporin, was effective in inhibiting eosinophil accumulation [18]. Although it is possible that rolipram and Org 20241 prevent the release of chemoattractants from T-cells, they may also be working through other mechanisms, such as inhibition of macrophage activation [21] or eosinophil locomotion [22]. There is also evidence for an effect of PDE IV inhibitors at the level of the pulmonary microvascular endothelium [23, 24].

PDE IV is a major cAMP-hydrolysing isoenzyme in all pro-inflammatory and immunocompetent cells such as T-lymphocytes [25], monocytes [26], neutrophils [27] and eosinophils [7, 28]. The T-lymphocyte also contains a significant amount of PDE III activity [8, 25]. PDE III or PDE IV inhibitors attenuate thymidine incorporation induced by phytohemagglutinin in T-lymphocytes, with a much greater inhibition achieved when they are used in combination [25]. More recent studies have shown that PDE IV inhibitors suppress IL-2, IL-4 and INF γ released from T-cells [29, 30] by a mechanism that may involve reduced gene transcription [31]. These effects were similarly enhanced by a PDE III inhibitor. These observations are in line with our *in-vivo* studies, although we have not shown at the doses we have chosen any greater effectiveness of the combined type III and type IV inhibitor, Org 20421. It is likely that we were using maximally effective doses.

We found that both rolipram and Org 20421 did not inhibit OA-induced acute signs of respiratory distress following allergen exposure. We did not measure lung function at this time-point and our study was not designed to examine the acute bronchoconstrictor response. However, there has been reports of rolipram inhibiting ovalbumin-induced bronchoconstriction in sensitised guinea-pigs [14]. Because rolipram minimally affected exogenous spasmogen-induced airway constriction [14], it has been proposed that rolipram may inhibit mast cell degranulation.

In summary, we have shown that inhibition of type IV and possibly type III PDE isoenzymes prevents eosinophil and neutrophil accumulation into the lungs of sensitised Brown-Norway rats induced by allergen challenge. These observations suggest that these inhibitors possess anti-inflammatory properties.

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